SUNTHORNTHEPVARAKUL et al. describe a clone that is transfected with a mutated form of the thyrotropin receptor and a luciferase reporter construct. More specifically, on page 156 under the heading "Construction of Wild-Type and Mutant Thyrotropin-Receptor cDNA Expression Vectors", it is expressly stated that vectors are generated expressing mutant forms of the receptor. Once the final constructs are completed, the mutated forms are verified by sequencing. Subsequently, these constructs and the luciferase reporter construct are transfected into the clone.

Under the heading "Functional Studies of the Thyrotropin Receptors in a Transient Transfection System", it is clearly stated that "Eight to 12 hours after transfection, the cells were washed and incubated for 48 hours with the complete medium in the absence or presence of various amounts of recombinant human thyrotropin". It follows that almost immediately following transfection, i.e. within days of transfection, experiments were undertaken. This is because the clones were not stably transfected with the said receptor or reporter construct. Rather, they were transiently transfected, so producing cells that can only be used once before being thrown away. Further, these transiently transfected cells are then used to measure the response of mutated receptors to their physiological hormone.

The above analysis is also true of the CLIFTON-BLIGH et al. publication. In this document under the heading "Functional Studies", it is stated that: "DNA fragments bearing each

mutation were replaced in full-length wild-type TSH receptor cDNA cloned into the eukaryotic expression vector pSVL, and constructs were verified by sequencing". Further, on page 1095 at the top of column 2, it is stated that "Twenty-four hours after transfection, the medium was replaced to include bovine TSH or recombinant human TSH, as appropriate. Sixteen hours later, cells were lysed and assayed for luciferase..." Here again, within a short time of transfection the experiments were undertaken. This is because the modified TSH receptor and the reporter construct were not stably transfected. This again means the cells could only be used once before being discarded. Further, the cells were only used to measure the response of mutated receptors to their physiological hormone.

In contrast to the above citations, the work described in the above patent application concerns the stable transfection of the wild-type version of the thyrotropin receptor and the luciferase reporter construct. Stable transfection of these elements is required because the above application concerns the development of an assay kit that can be used some time after its development, i.e. anytime, anywhere, and moreover, repetitively. Further, the assay kit has been developed to measure pathological autoantibodies of the thyrotropin receptors. This is in contrast to the above two citations wherein experiments are undertaken to measure the response of mutated receptors to their physiological hormone. Measurements of pathological autoantibodies involves measurement of autoantibodies to the thyrotropin receptor. Our assay kit therefore involves the production of a stably

transfected clone which can be transported to a site of interest and then used repeatedly to undertake pathological studies, i.e. to measure autoantibodies to wild-type thyrotropin receptor and so provide an assay that can monitor Grave's Disease, (wherein the autoantibody is agonistic) or auto-immune hypothyroidism (where the antibody is antagonistic).

The Examiner will appreciate that the prior art documents tend to teach away from the subject matter of the above application insofar as they teach for the transient transfection of mutant thyrotropin receptors into a cell clone whereby almost immediate experimentation must be performed to measure the response of mutated receptors to various agents. Further, because the transfection is transient these experiments cannot be repeated.

The content of the present application goes against this teaching in that it concerns the development of a stable assay using wild-type receptor which can be used to measure one of two pathologies anytime, anywhere and repeatedly.

In light of the above discussion, therefore, it is believed that the rejection of the pending claims for anticipation based on either of the two applied references, should now be withdrawn.

In view of the present amendment and the foregoing remarks, therefore, it is believed that this application is now in condition for allowance, with claims 36, 38-40, 42, 44 and 45, as amended. Allowance and passage to issue on that basis are accordingly respectfully requested.

Attached hereto is a marked-up version of the changes made to the claims. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Respectfully submitted,

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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

IN THE CLAIMS:

Claim 36 has been amended as follows:

--36. (thrice amended) A clone [expressing human TSH-R (thyotropin receptor)] stably transfected with a gene expressing wild-type human thyrotropin receptor and a reporter construct comprising cDNA of both

(i) a reactant capable of causing a measurable response when brought into contact with a corresponding substrate, and

(ii) a promoter [comprising no more than two] containing cAMP response elements (CREs), comprising a promoter sequence or synthetic oligonucleotide which contains the CRE consensus sequence, TGACGTCA,

whereby cAMP levels vary with expression of the reactant [vary with induced endogenous cAMP levels]; and wherein the promoter sequence or synthetic oligonucleotide is that for the glycoprotein hormone alpha subunit that contains a tandem repeat of the CRE consensus sequence, TGACGTCA.--

Claim 40 has been amended as follows:

--40. (thrice amended) cDNA or mRNA [expressing human TSH-R (thyotropin receptor)] stably transfected with a gene expressing wild-type human thyrotropin receptor and a reporter construct comprising cDNA of both

(i) a reactant capable of causing a measurable response when brought into contact with a corresponding substrate, and

(ii) a promoter [comprising no more than two] containing cAMP response elements (CREs), comprising a promoter sequence or synthetic oligonucleotide which contains the CRE consensus sequence, TGACGTCA,

whereby cAMP levels vary with expression of the reactant [vary with induced endogenous cAMP levels]; and wherein the promoter sequence or synthetic oligonucleotide is that for the glycoprotein hormone alpha subunit that contains a tandem repeat of the CRE consensus sequence, TGACGTCA.--